

## **The effect of central stimulant drugs on acetylcholine release from rat cerebral cortex**

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1. The effects of central stimulant drugs injected intraperitoneally were examined on the release of acetylcholine (ACh) from the cerebral cortex of the anaesthetized rat. The effects of the drugs in increasing ACh release were approximately parallel to the increases produced in the electrical activity of the brain.
  2. Leptazol in a dose of 150 mg/kg increased the release of ACh by 2.9 times the resting release and in a dose of 300 mg/kg by 7.5 times ; on the e.e.g. the injection produced a large increase in the electrical activity.
  3. Picrotoxin in a dose of 12 mg/kg increased the release of ACh by 7.5 times and on the e.e.g. caused a large increase in activity.
  4. Strychnine in doses of 8 mg/kg and 12 mg/kg increased the release of ACh by 2.0 and 2.4 times and produced a small increase in the activity of the e.e.g.
  5. Dexamphetamine in a dose of 100 mg/kg increased the release of ACh by 1.9 times and had no appreciable effect on the e.e.g.
  6. Nikethamide in a dose of 2 g/kg increased the release of ACh by 2.3 times and produced no appreciable change in the e.e.g.
  7. Caffeine in a dose of 100 mg/kg produced no significant effect on the release of ACh.
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In the present experiments central stimulant drugs with different sites of action in the neuraxis were used to investigate, in the anaesthetized rat, the relationship between increased neuronal activity in the brain produced by drugs and release of acetylcholine (ACh) from the cerebral cortex.

Experiments on anaesthetized animals have shown that in the presence of an anticholinesterase, ACh is released from the cerebral cortex (MacIntosh & Oborin, 1953 ; Mitchell, 1963). The ACh originates from cholinergic nerve terminals of ascending fibres, the cell bodies of which appear to be located in the thalamic nuclei and the reticular formation of the brain stem (Shute & Lewis, 1963 ; Collier & Mitchell, 1966). The rate of release of ACh from the sensory cortex is dependent

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on neuronal activity and can be increased by stimulation of the appropriate sensory pathways (Mitchell, 1963 ; Collier & Mitchell, 1966 ; Neal, Hemsworth & Mitchell, 1968). Similarly stimulation of the mesencephalic reticular formation produces a generalized increase in release of ACh from the cerebral cortex (Kanai & Szerb, 1965 ; Celesia & Jasper, 1966).

Mitchell (1963) showed in sheep that leptazol increased and deep pentobarbitone sodium anaesthesia decreased the release of ACh from the cerebral cortex. These changes in ACh release roughly paralleled the changes in electrical activity of the brain.

## Methods

Male Wistar rats (weight 250–350 g) were anaesthetized by an intraperitoneal injection of Dial compound (allobarbitone and urethane ; Ciba Ltd., 0.85 ml./kg) and a tracheotomy was performed. The method of collecting the ACh released from the brain was similar to that introduced by MacIntosh & Oborin (1953) and described in detail by Mitchell (1963). The cerebral cortex was widely exposed bilaterally. The dura mater was removed and a Perspex cylinder was placed on the somatosensory area of each cerebral hemisphere. Leakage from the cylinders was prevented by using soft paraffin as a seal. The head of the animal and the collecting cylinders were fixed as illustrated in Fig. 1. The body temperature was maintained at 37° C ( $\pm 1^\circ$  C) by an automatically controlled electric blanket (Electrophysiological Instruments Ltd.).

Both collecting cylinders were filled with 1.0 ml. of Ringer-Locke solution (NaCl 9.0, KCl 0.42,  $\text{CaCl}_2$  0.24,  $\text{NaHCO}_3$  0.2, glucose 2.0 g/l.) prewarmed to 37° C. This solution contained eserine sulphate ( $10^{-4}$  g/ml.) and in most experiments atropine sulphate ( $4.0 \times 10^{-7}$  g/ml.) as well. The solutions were bubbled with a gas mixture containing 95% oxygen and 5% carbon dioxide and were left in contact with the cortex for a 30 min collection period ; the solution was then replaced with fresh Ringer-Locke. The first sample from each cup was rejected ; the subsequent 30 min samples were assayed for ACh on the dorsal muscle of the leech (Collier & Mitchell, 1966). The samples from each cerebral hemisphere were assayed individually. The results are expressed in terms of the resting release of ACh obtained before drug administration. Each value obtained after drug administration was divided by the average resting release obtained from the two collecting periods immediately preceding injection of the drug. This procedure was adopted

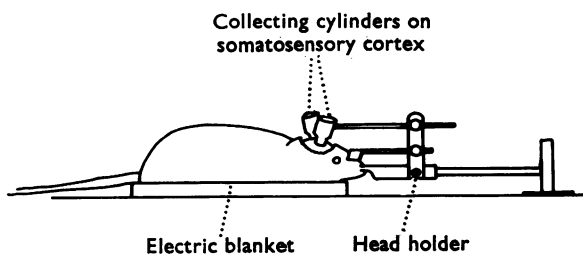


FIG. 1. Method of collecting ACh released from cerebral cortex of the anaesthetized rat.

in order to compare results obtained from different animals in which the absolute amounts of ACh release varied considerably.

In some experiments arterial blood pressure was recorded through a polythene cannula inserted into a carotid artery and connected to a Condon mercury manometer. In these experiments the electroencephalogram (e.e.g.) was recorded as well, leads being taken from needles inserted in the parietal bones.

**Drugs used.** Strychnine hydrochloride (British Drug Houses), picrotoxin (British Drug Houses), pentamethylenetetrazole (leptazol, L. Light), nikethamide (Anacardone, British Drug Houses), dexamphetamine sulphate (Smith, Kline & French). The drugs were injected intraperitoneally and the doses are expressed in terms of the salts.

## Results

The resting release of ACh from the cerebral cortex varied considerably in different rats, but variations in release from any single rat were small and did not exceed  $\pm 20\%$  of the mean value obtained during ten collection periods (5 hr). Differences in release between cerebral hemispheres of the same rat were usually small. With atropine present in the collecting solution the resting release of ACh varied from 1.7 to 9.7 ng/30 min/0.2 cm<sup>2</sup> cortex. In the absence of atropine the spontaneous release was a fifth to one quarter of this amount.

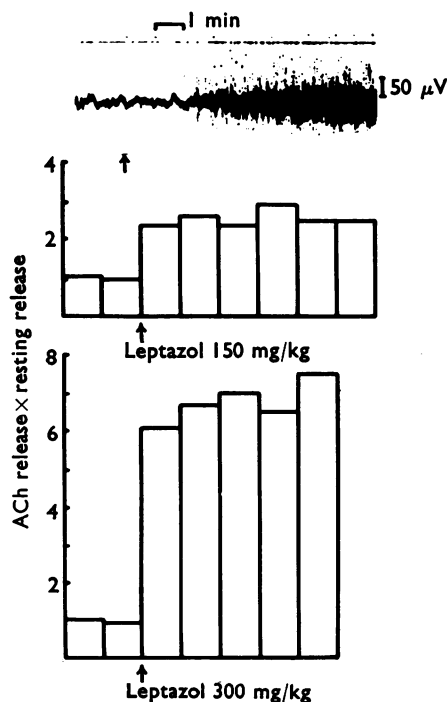


FIG. 2. Effect of leptazol (150 mg/kg and 300 mg/kg) on the mean ACh release from cerebral cortex of eight anaesthetized rats. Each block represents a 30 min collection period. Top panel, e.e.g. from one experiment with 150 mg/kg.

*Leptazol*. As shown in Fig. 2, a dose of 150 mg/kg increased the mean release of ACh 2.9 times (eight rats) and a dose of 300 mg/kg, 7.5 times (eight rats) the resting release. Figure 2 also illustrates that the increased release was nearly maximal during the first 30 min period of collection after the injection and did not appreciably decrease over a period of 2.5–3 hr. This increase in release of ACh was associated with increased electrical activity of the cerebral cortex. In two experiments in which the arterial blood pressure was recorded, the injection of 150 mg/kg produced, after a transient fall of 12 mm, a rise of 30 and 45 mm Hg. This was followed by a gradual return to normal which took approximately 60 and 80 min.

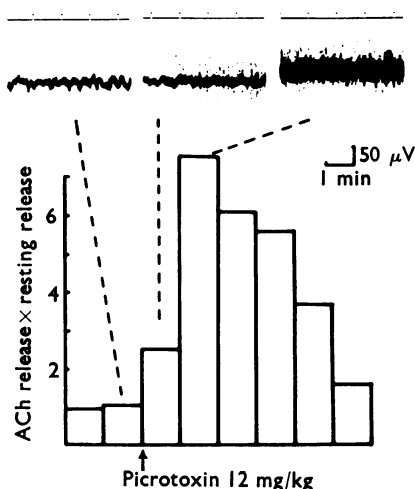


FIG. 3. Effect of picrotoxin (12 mg/kg) on mean ACh release from cerebral cortex of six anaesthetized rats. Top panel, e.e.g. from one experiment.

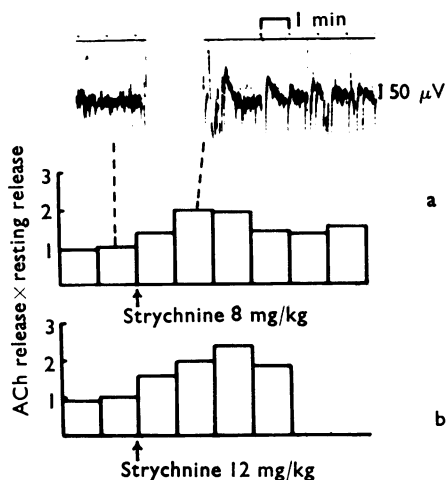


FIG. 4. Effect of strychnine (8 mg/kg and 12 mg/kg) on the mean release of ACh from cerebral cortex of six anaesthetized rats. Top panel, e.e.g. from one experiment with 8 mg/kg.

**Picrotoxin.** As shown in Fig. 3 a dose of 12 mg/kg raised the mean release of ACh from six rats by 7.5 times the resting release. The increased release was maximal in the second 30 min period, and had almost disappeared 3 hr after the injection. The change in e.e.g. went parallel to the increase in ACh release from the cerebral cortex; increased electrical activity was maximal during the second 30 min period and had almost disappeared after 3 hr. Figure 3 shows for one experiment the e.e.g. before and during the first and second 30 min collection periods after the injection. In two experiments in which arterial blood pressure was recorded, picrotoxin produced the same changes as described for leptazol.

**Strychnine.** An intraperitoneal injection of strychnine (4 mg/kg) produced convulsions but did not increase the rate of release of ACh from the brain. On the other hand a dose of 8 mg/kg increased the mean release of ACh in six experiments by twice the resting release as shown in Fig. 4a. The maximum release occurred during the second 30 min collection period after the injection. A dose of 12 mg/kg increased the mean ACh release from six animals by 2.4 times the resting release (Fig. 4b); these six animals died between 2 and 3 hr after the injection. In two rats in which the injection of 8 mg/kg was examined on the arterial blood pressure and e.e.g. there was an increase in the electrical activity of the brain and on the blood pressure a transient fall of 10 mm Hg followed by a rise of approximately 20 mm Hg above the pre-injection level; the blood pressure returned to the initial level after 30 and 42 min.

**Dexamphetamine.** This non-convulsant drug, when given intraperitoneally to nine rats in a dose of 100 mg/kg, increased the mean release of ACh by 1.9 times the resting release. The maximum release occurred during the second 30 min collecting period after the injection and the effect disappeared after 150 min. These results are illustrated in Fig. 5a. In one rat, in which the effect of dexamphetamine (100 mg/kg) was examined on the arterial blood pressure and e.e.g., it produced a rise of 80 mm Hg lasting 1 hr but no obvious change in the e.e.g.

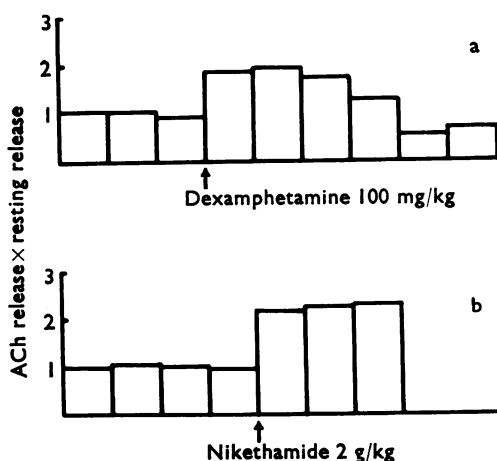


FIG. 5. Effect of dexamphetamine (100 mg/kg) and of nikethamide (2 g/kg) on the mean ACh release from cerebral cortex of nine and six anaesthetized rats.

*Nikethamide.* In sub-lethal doses (0.5–1 g/kg) nikethamide had no appreciable effect on release of ACh from the brain. A dose of 2 g/kg injected into six rats produced death of all the animals after 90 to 120 min but during the survival period the mean ACh release increased 2.3 times, as illustrated in Fig. 5b. In one experiment in which the effect of this dose was examined on the e.e.g. and arterial blood pressure, no appreciable changes were observed.

*Caffeine.* An injection of 100 mg/kg produced no significant effect on cortical ACh release.

## Discussion

The present experiments show that the effect of intraperitoneal injections of central stimulant drugs on release of ACh from the cerebral cortex is approximately in parallel with changes in the electrical activity of the brain observed in the e.e.g. Thus leptazol and picrotoxin which produced large increases in central ACh release also caused the greatest increase in electrical activity of the brain. Strychnine, dexamphetamine and nikethamide which were less effective in increasing the release of ACh had only a small or no effect on the e.e.g. of anaesthetized rats. On the other hand there was no definite correlation between increased ACh release and convulsive activity for the most potent convulsant investigated, strychnine, was relatively ineffective in increasing ACh release. This suggests that the effectiveness of drugs in increasing release of ACh from the cerebral cortex is associated with their specific sites of action.

The increased central release of ACh produced by drugs is most likely due to increased neuronal activity of cholinergic pathways in the brain. An important ascending cholinergic pathway originates in the mid-brain reticular formation and terminates in the cerebral cortex. This pathway passes through the striatum and septum and may be responsible for arousal responses and desynchronization of the e.e.g. (Kanai & Szerb, 1965; Phillis & Chong, 1965; Celesia & Jasper, 1966; Krnjević, 1967). Another ascending cholinergic pathway originating in the specific nuclei of the thalamus suggested by Collier & Mitchell (1966), is probably associated with after-discharges in the cerebral cortex (Brownlee & Mitchell, 1968). Thus a drug which stimulates either or both of these systems, directly or indirectly, might be expected to evoke an increase in the release of ACh from the cerebral cortex.

The increased ACh release produced by leptazol confirms findings of Mitchell (1963), Beleslin, Polak & Sproull (1965) and Celesia & Jasper (1966). The large increase in ACh release caused by leptazol was accompanied by a correspondingly large increase in the electrical activity of the brain. In view of the widespread action of leptazol in the brain it seems most likely that this drug stimulates both ascending cholinergic systems but probably also the small proportion of intra-cortical cholinergic fibres which are known to exist (Krnjević, 1967), because the higher centres are more sensitive to the action of leptazol than those of the medulla and pons (Jolly & Steinhaus, 1956), and because it is believed that convulsions originate largely from the most sensitive areas—the sub-cortex and cortex (Biehler, 1940).

Picrotoxin, another potent convulsant, also had a powerful effect in increasing ACh release and electrical activity of the brain. It is known to have a widespread

central action but not as widespread as leptazol and there is evidence from experiments on frogs (Schriever & Perschmann, 1935) that the mid-brain is the primary site of action of this drug. This is supported by experiments which show that picrotoxin increases glycogen content in brainstem neurones (Chance, 1951). The large increase in central ACh release is probably caused, as with leptazol, by excitation of cholinergic neurones originating in the reticular formation and in the thalamus.

Strychnine, the most potent convulsant drug used in the present experiments, acts primarily on the spinal cord. This would explain why it was relatively ineffective in increasing ACh release, even in high convulsive doses. These results are in agreement with those of Celesia & Jasper (1966), who applied strychnine topically to the brain of the cat and obtained only a 50% increase in ACh release from the cortex even when generalized strychninization with widespread discharges had occurred. The results differ from those of Beleslin *et al.* (1965), however, who obtained with intravenous strychnine a large increase in the ACh release from the parietal cortex of the cat. Their different result may be due to the use of the intravenous route of administration of strychnine in contrast to intraperitoneal injection or topical application, but this would not explain why the ACh release was so much smaller in these two conditions in spite of the relatively strong effect on the electrical activity of the brain.

Dexamphetamine seems to increase the release of ACh by exciting cell bodies of ascending cholinergic fibres in the midbrain reticular formation because intravenous amphetamine produces an arousal type of e.e.g. (Hiebel, Bonvallet, Huvé & Dell, 1954 ; Bradley & Elkes, 1957 ; Longo & Silvestrini, 1957) and this desynchronizing action is abolished by midbrain lesions which destroy the mesencephalic reticular formation (Killam, Gangloff, Konigsmark & Killam, 1959) or interrupt its pathway to the cortex (Hiebel *et al.*, 1954). The effect of dexamphetamine on the release of ACh was rather small compared with the five to six-fold increase produced by Kanai & Szerb (1965) on electrical stimulation of the reticular formation in cats. In the present experiments the intraperitoneal injection of dexamphetamine, however, produced also little increase in the electrical activity of the brain. On the other hand an intravenous infusion of dexamphetamine (5 mg/kg) in rabbits was found to increase ACh release from the cerebral cortex up to ten-fold and this increase was associated with e.e.g. desynchronization (unpublished results). The different results obtained in rats and rabbits are probably not the result of species differences but of the different routes of administration used.

Dexamphetamine produced a larger rise in blood pressure than any of the other convulsants examined but caused a relatively small increase in ACh release compared with leptazol and picrotoxin, so changes in blood pressure are not responsible for the increases in release of ACh. This conclusion is in accord with Ingvar (1958) that an increase in cerebral blood flow does not initiate an arousal response.

Nikethamide has a mixed stimulant and depressant action on the brain but its stimulant action is not of cortical origin ; like picrotoxin, it affects subcortical regions (Ten Cate & Swijgman, 1945). Its relatively weak action in increasing ACh release, which was not associated with increased activity of the e.e.g., is thus probably due to a rather weak direct or indirect action on the reticular formation and/or on the thalamus.

Because caffeine produces e.e.g. arousal (Shallek & Kuehn, 1959) but does not significantly increase ACh release, it may act on non-cholinergic cortical neurones, possibly on the cholinceptive cells in layer five of the cerebral cortex. A similar type of action is found with topically applied neostigmine which is capable of initiating local desynchronization followed by epileptic activity in the cortex without increasing ACh release (Celesia & Jasper, 1966).

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